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(71) Applicant (for all designated States except US): FISONS PLC
[GB/GB]; Fison House, Princes Street, Ipswich, Suffolk IP1
1QH (GB).

(72) Inventor; and

(75) Inventor/Applicant (for US only): GODDARD, Nicholas, John
[GB/GB]; 15 Lowfield Gardens, Glazebury, Warrington
WA3 5LY (GB).

(74) Agent: JONES, Stephen, Anthony; E N Lewis & Taylor, 144
New Walk, Leicester LE1 7JA (GB).

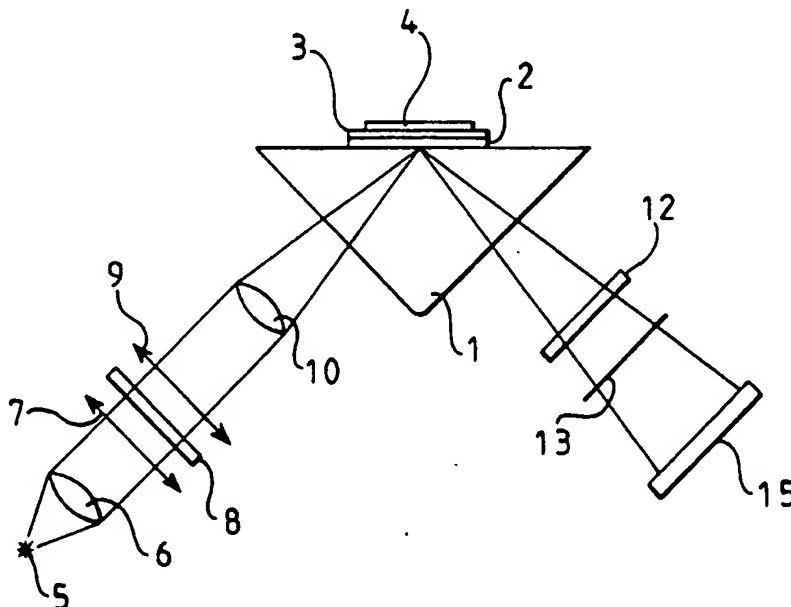
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(54) Title: ANALYTICAL DEVICE



(57) Abstract

Apparatus for the determination of a chemical or biochemical species comprises a resonant optical sensor (1-4) disposed in a light path between a polychromatic light source (5) and a detector (15). The sensor (1-4) is irradiated with light from the light source (5) at a range of angled simultaneously, and light reflected from the sensor (1-4) is allowed to reach the detector (15) only when resonance occurs. The intensity of the polychromatic light from the light source (5) is modulated as a function of frequency, preferably periodically.

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Title : Analytical Device

This invention relates to sensors, especially those termed biosensors, ie devices for the analysis of biologically active species such as antigens and antibodies in samples of biological origin. In particular, the invention relates to biosensors based on resonant optical phenomena, eg surface plasmon resonance or resonant attenuated or frustrated total internal reflection.

Many devices for the automatic determination of biochemical analytes in solution have been proposed in recent years. Typically, such devices (biosensors) include a sensitised coating layer which for optical devices might be located in the evanescent region of a resonant field. Detection of the analyte typically utilizes optical techniques such as, for example, surface plasmon resonance (SPR), and is based on changes in the thickness and/or refractive index of the coating layer resulting from interaction of that layer with the analyte. This causes a change, eg in the angular position of the resonance.

Other optical biosensors include a waveguide in which a beam of light is propagated. The optical characteristics of the device are influenced by changes occurring at the surface of the waveguide. One form of optical biosensor is based on frustrated total reflection. The principles of frustrated total reflection (FTR) are well-known; the technique is described, for example, by Bosacchi and Oehrle [Applied Optics (1982), 21, 2167-2173]. An FTR device for use in immunoassay is disclosed in European Patent Application No 2205236A and comprises a cavity layer bounded on one side by the sample under investigation and on the other side by a spacer layer which in turn is mounted on a substrate. The substrate-spacer layer interface is irradiated with monochromatic radiation such that total reflection occurs, the associated evanescent field penetrating through the spacer layer. If the thickness

of the spacer layer is correct and the incident parallel wave vector matches one of the resonant mode propagation constants, the total reflection is frustrated and radiation is coupled into the cavity layer. The cavity layer must be composed of material which has a higher refractive index than the spacer layer and which is transparent at the wavelength of the incident radiation.

SPR could be thought of as a special case of FTR. However, for present purposes a distinction is drawn between devices in which energy is coupled into a metal layer (SPR devices) and devices in which radiation is coupled into a dielectric layer (FTR devices).

In SPR devices there is a dip in the reflected light intensity on resonance. Rather than detecting a dark band against a light background, there are certain advantages in detecting a bright band against a dark background and this can be achieved using the method and apparatus disclosed in International Patent Application WO 92/04617. In devices based on the principle of FTR, on the other hand, the overall intensity of the reflected light is substantially unchanged. The light does, however, undergo a phase change at resonance and, by appropriate use of polarisers, resonance can be detected as a bright line on a dark background, or vice versa.

In both SPR and FTR, two basic measurement approaches are possible: irradiating with monochromatic light simultaneously or sequentially at a range of angles, or using polychromatic light at a fixed angle of incidence. The former approach is usually preferred, especially when irradiation occurs simultaneously over a range of angles using a wedge-shaped incident beam. This is described, in relation to SPR, in European Patent Application No 0305109A.

A disadvantage of known SPR and FTR devices of the kind described is that unavoidable variations, due to manufacturing

tolerances and the like can mean that the position of the initial resonance varies quite widely from device to device. To accommodate this variation, the angular range of the detector must therefore be much wider than the range over which the position of the resonance shifts during measurements. This increases the cost and complexity of the system, as well as increasing the data processing requirements without concomitant benefit since the majority of the data recorded during each measurement will not contribute to the analysis of the results.

There has now been devised an apparatus involving the use of a resonant optical sensor for the determination of a chemical or biochemical species, which overcomes or substantially mitigates some or all of the disadvantages of the prior art arrangements described above.

According to the invention, there is provided apparatus for the determination of a chemical or biochemical species, comprising a resonant optical sensor disposed in a light path between a polychromatic light source and a detector, there being provided means for irradiating the sensor with light from the light source at a range of angles simultaneously, and the arrangement being such that light reflected from the sensor is allowed to reach the detector only when resonance occurs, and the intensity of the polychromatic light from the light source is modulated as a function of frequency.

The apparatus according to the invention is advantageous primarily in that the intensity modulation applied to the light input to the sensor as a function of frequency is mirrored as a corresponding modulation of the detected light intensity as a function of angle. All of the data accumulated by the detector may then be used to monitor the shift in phase of this modulation which occurs during measurement. The general form of the modulation applied to the input light with frequency is the same, or substantially the same, as the

modulation of the detected light with angle. If, as is generally the case, the form of that modulation is known, then the whole of the detected data may be used in the analysis. In addition, a smaller detector may be used than in the prior art. Also, as described more fully below, the components necessary to provide the polychromatic light and to generate the modulation are relatively simple, and the form of the modulation may be chosen at will.

Many different forms of modulation of the input light may be employed. Periodic modulation is preferred. For ease of generation and data analysis, a sine wave modulation is particularly preferred. Where the modulation is between a maximum value and zero, it is more properly described as a sine^2 modulation.

A sine^2 wave modulation may be generated simply by passing polarised input light through a so-called phaseplate and a polariser. In the present case, for light with a wavelength of the order of 600nm, a sine^2 intensity modulation with a period of around 20nm can be produced using a crystal quartz plate with a thickness of the order of 1mm. Such a multiple order phaseplate can be readily manufactured and is sufficiently robust for routine use. The period of the sine wave modulation may be simply changed by changing the thickness of the quartz plate.

For typical applications, the bandwidth of the polychromatic light may be around 200nm, say 800-600nm. Any broad band light source may be used, eg a tungsten-filament lamp or an arc lamp.

Simultaneous irradiation over a range of angles is achieved simply by forming the input beam into a wedge shape, in a conventional manner. Typically, the range of angles over which irradiation occurs is 15° or so.

The data recorded by the detector, which is an array of measured intensities as a function of angle, may be analysed in various ways. For example, the data may be fitted to a known function, eg a sine wave-type function. The change in phase of the wave provides an indication of the extent of chemical or physico-chemical events at the sensor surface. Alternatively, the data may be subjected to a Fourier Transformation, eg using a suitable Fast Fourier Transform algorithm. Such a transformation provides information not only about the period of an oscillation, but also its phase.

In a further alternative, a reference sample may be monitored simultaneously with the sample under investigation and a comparison made of the results obtained from both. In particular, the shift in the reference data may be calculated which is necessary to obtain the best fit with the measured data for the sample under investigation.

In another embodiment, useful particularly where the period of the output modulation is small compared with the measured shift in resonance, there are provided one or more point detectors at fixed points. These are used to monitor the number of peaks in the measured signal passing over them as the output signal shifts during measurement. It is preferred to use at least two such fixed point detectors, located at different points on the cycle of the modulated output signal. This enables shifts of the output signal in opposite directions to be distinguished.

The sensor may be an FTR sensor. Such a sensor will generally include an optical structure comprising

- a) a cavity layer of transparent dielectric material of refractive index n_1 ,
- b) a dielectric substrate of refractive index n_2 , and
- c) interposed between the cavity layer and the substrate, a dielectric spacer layer of refractive index n_3 .

In use, the interface between the substrate and the spacer layer is irradiated with light such that internal reflection occurs. Resonant propagation of a guided mode in the cavity layer will occur, for a given wavelength, at a particular angle of incidence of the exciting radiation. Where, as in the present invention, polychromatic light is used, resonance for one wavelength will occur at one angle of incidence, and for a different wavelength at a different angle of incidence. Since the intensity of the input radiation is modulated as function of frequency, the intensity of the detected radiation will be modulated in a similar manner as a function of angle.

The angular position of the resonant effect depends on various parameters of the sensor device, such as the refractive indices and thicknesses of the various layers. In general, it is a pre-requisite that the refractive index n_c of the cavity layer and the refractive index n_s of the substrate should both exceed the refractive index n_p of the spacer layer. Also, since at least one mode must exist in the cavity to achieve resonance, the cavity layer must exceed a certain minimum thickness.

The cavity layer is preferably a thin-film of dielectric material. Suitable materials for the cavity layer include zirconium dioxide, titanium dioxide, aluminium oxide and tantalum oxide.

The cavity layer may be prepared by known techniques, eg vacuum evaporation, sputtering, chemical vapour deposition or in-diffusion.

The dielectric spacer layer must have a lower refractive index than both the cavity layer and the substrate. The layer may, for example, comprise an evaporated or sputtered layer of magnesium fluoride. Other suitable materials include lithium fluoride and silicon dioxide. Apart from the evaporation and sputtering techniques mentioned above, the spacer layer may be

deposited on the substrate by a sol-gel process, or be formed by chemical reaction with the substrate.

The sol-gel process is particularly preferred where the spacer layer is of silicon dioxide.

The refractive index of the substrate (n_1) must be greater than that (n_2) of the spacer layer but the thickness of the substrate is generally not critical.

By contrast, the thickness of the cavity layer must be so chosen that resonance occurs within an appropriate range of coupling angles. The spacer layer will typically have a thickness of the order of several hundred nanometres, say from about 200nm to 2000nm, more preferably 500 to 1500nm, eg 1000nm. The cavity layer typically has a thickness of a few tens of nanometres, say 10 to 200nm, more preferably 30 to 150nm, eg 100nm.

It is particularly preferred that the cavity layer has a thickness of 30 to 150nm and comprises a material selected from zirconium dioxide, titanium dioxide, tantalum oxide and aluminium oxide, and the spacer layer has a thickness of 500 to 1500nm and comprises a material selected from magnesium fluoride, lithium fluoride and silicon dioxide, the choice of materials being such that the refractive index of the spacer layer is less than that of the cavity layer.

Preferred materials for the cavity layer and the spacer layer are tantalum oxide and silicon dioxide respectively.

At resonance, the incident light is coupled into the cavity layer by FTR, propagates a certain distance along the cavity layer, and couples back out (also by FTR). The propagation distance depends on the various device parameters but is typically of the order of 1 or 2mm. At resonance the reflected light will undergo a phase change.

If the sensor is an SPR device it will comprise a dielectric medium, eg a glass prism, on which is coated a thin layer of metal. Oscillation of the surface-plasma of free electrons which exists at the metal-dielectric boundary is affected by the refractive index of the material adjacent to the metal surface. For a given wavelength, resonance occurs when the angle of incidence of the radiation has a particular value, and this value is dependent on the refractive index of the material adjacent to the metal. Thus, changes in this refractive index give rise to changes in the angle at which resonance occurs.

A variety of metal coatings may be applied to the glass prism. Most usually, the metal coating is of silver.

In normal SPR, only TM-polarised (or p-polarised) light is used, since TE-polarised (or s-polarised) light cannot excite SPR by the total internal reflection process. Resonance is detected as a dip in light intensity due to absorbance by the metal layer at resonance. In the present invention, however, it is necessary for light to reach the detector at resonance but not otherwise. This can be achieved by using the variation on the SPR technique described in International Patent Application WO 92/04617. In this technique, the incident radiation contains both TE-polarised and TM-polarised components and a polarisation analyser is interposed between the sensor and the detector such that, away from resonance, little or no light reaches the detector.

For use in the determination of biochemical species, the surface of the sensor, ie the surface of the cavity layer in the case of an FTR sensor or the surface of the metal layer in SPR, will generally be sensitised by having biomolecules, eg specific binding partners for the analyte(s) under test, immobilised upon it. The immobilised biochemicals may be covalently bound to the sensor surface by methods which are well known to those skilled in the art.

The invention will now be described in more detail, by way of illustration only, with reference to the accompanying drawings in which

Figure 1 is a schematic view (not to scale) of an apparatus according to the invention,

Figure 2 depicts the intensity of the input light as a function of wavelength, and

Figure 3 depicts the intensity of the detected light as a function of angle of incidence.

Referring first to Figure 1, an FTR biosensor comprises a glass prism 1 coated over an area of its base with a first coating 2 of magnesium fluoride and a second coating 3 of titanium dioxide. The prism 1 and first and second coatings 2,3 together constitute a resonant optical structure, the first coating 2 acting as a spacer layer and the second coating 3 as a cavity layer. The first coating 2 has a thickness of approximately 1000nm and the second coating 3 a thickness of approximately 100nm.

Immobilised on the surface of the second coating 3 is a layer 4 of immobilised biochemicals, which act as specific binding partner for the analyte under test.

The interface between the base of the prism 1 and the first coating 2 is irradiated by a beam of polychromatic light from a tungsten-filament lamp 5. Light from the lamp 5 is collimated by a lens 6 and then passes through a first polariser 7, a phaseplate 8 and a second polariser 9. The light is then formed into a wedge-shaped input beam by a focussing lens 10. The sensor is thus irradiated with polychromatic light with a range of angles of incidence simultaneously. The range of angles is approximately 15°.

The first polariser 7 and the second polarizer 9 are arranged at 45° to the plane of incidence. Light incident on the biosensor is therefore linearly polarised with two equal components : transverse electric (TE) and transverse magnetic (TM).

The phaseplate 8 is a sheet of quartz approximately 1mm in thickness. The phaseplate 8 is oriented with its crystallographic axes at 45° to the plane of polarisation of the first polarizer 7 and the second polarizer 9. The effect of the phaseplate 8 and the second polarizers 9 is to modulate the intensity of the input light as a function of frequency. A sine² wave modulation is applied to the frequency spectrum of the input light, as shown in Figure 2. The modulation has a period of about 20nm.

All the light incident on the interface between the base of the prism 1 and the first coating 2 is reflected. For each angle of incidence, resonance occurs for a incident radiation of a particular wavelength.

Resonance is detected as a change of phase of the reflected light. The reflected light passes through a compensator 12 to a polarisation analyser 13. The compensator 12, which may be of any conventional form, is manually adjusted to remove any phase difference which is introduced into the TE and TM components on reflection and by birefringence in the optical path.

The analyser 13 is arranged at 90° to the first and second polarisers 7,9. The TE and TM components are interfered at the analyser to allow the phase change to be detected. Off resonance both components undergo a similar phase shift on reflection and the relative phase between the components is adjusted by the compensator 12 to give zero transmission through the analyser 13. This will apply for all angles except near resonance. Near resonance of either component,

the phase shift between the TE and TM components will vary rapidly with angle, resulting in maximum throughput of light through the analyser 13 at resonance.

Light passing through the analyser 13 is detected by a detector 15 comprising an array of charge coupled devices (CCD).

The measured light intensity is modulated as a function of angle in the same way as the input light intensity is modulated as a function of wavelength, as shown in Figure 3.

When the layer of immobilised biochemicals 4 is contacted with a sample containing the analyte under test, specific binding occurs between the biochemicals and the analyte molecules, resulting in a change in the refractive index in the vicinity of the surface of the device. This in turn results in a shift in the position of the resonance for each incident wavelength. Figure 3 shows a plot of the measured signal intensity against angle of incidence before and (dotted line) after complexation of the immobilised biochemicals with the analyte. As can be seen, the whole measured signal is shifted one way or the other by the complexation reaction.

The measured signal may be analysed in several ways. For instance, the signal may be fitted using conventional fitting algorithms to a sine-wave function (or a modified sine-wave function to take account of any distortions which occur). Alternatively, the signal may be subjected to a Fourier transformation. In any event, all of the accumulated data points are used to assess the extent to which the signal is shifted left or right.

Claims

1. Apparatus for the determination of a chemical or biochemical species, comprising a resonant optical sensor disposed in a light path between a polychromatic light source and a detector, there being provided means for irradiating the sensor with light from the light source at a range of angles simultaneously, and the arrangement being such that light reflected from the sensor is allowed to reach the detector only when resonance occurs, and the intensity of the polychromatic light from the light source is modulated as a function of frequency.
2. Apparatus as claimed in Claim 1, wherein a periodic modulation is applied to the polychromatic light.
3. Apparatus as claimed in Claim 2, wherein the modulation is a sine² modulation.
4. Apparatus as claimed in Claim 3, wherein the sine² modulation is generated by passing polarised input light through a phaseplate and a polariser.
5. Apparatus as claimed in Claim 4, wherein the phase plate is a crystal quartz plate with a thickness of the order of 1mm.
6. Apparatus as claimed in any preceding claim, wherein the sensor is an FTR sensor including an optical structure comprising
 - a) a cavity layer of transparent dielectric material of refractive index n_1 ,
 - b) a dielectric substrate of refractive index n_1 , and
 - c) interposed between the cavity layer and the substrate, a dielectric spacer layer of refractive index n_2 .
7. Apparatus as claimed in Claim 6, wherein the cavity layer

is a thin film of dielectric material.

8. Apparatus as claimed in Claim 7, wherein the spacer layer has a thickness of from about 200nm to 2000nm, and the cavity layer has a thickness of 10 to 200nm.

9. Apparatus as claimed in any one of Claims 1 to 5, wherein the sensor is an SPR device comprising a dielectric medium on which is coated a thin layer of metal.

10. Apparatus as claimed in Claim 9, wherein the metal is silver.

11. Apparatus as claimed in Claim 9 or Claim 10, wherein the incident radiation contains both TE-polarised and TM-polarised components and a polarisation analyser is interposed between the sensor and the detector such that, away from resonance, little or no light reaches the detector.

12. Apparatus as claimed in any preceding claim, wherein the sensor is a biosensor for use in the determination of biochemical species, and a surface of the sensor is sensitised by having biomolecules immobilised upon it.

13. A method of determining a chemical or biochemical species in a sample, which method comprises the steps of

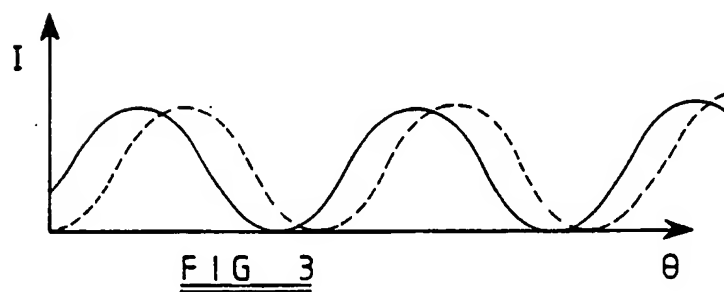
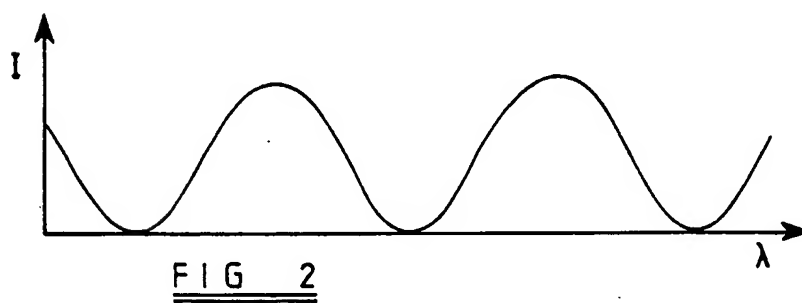
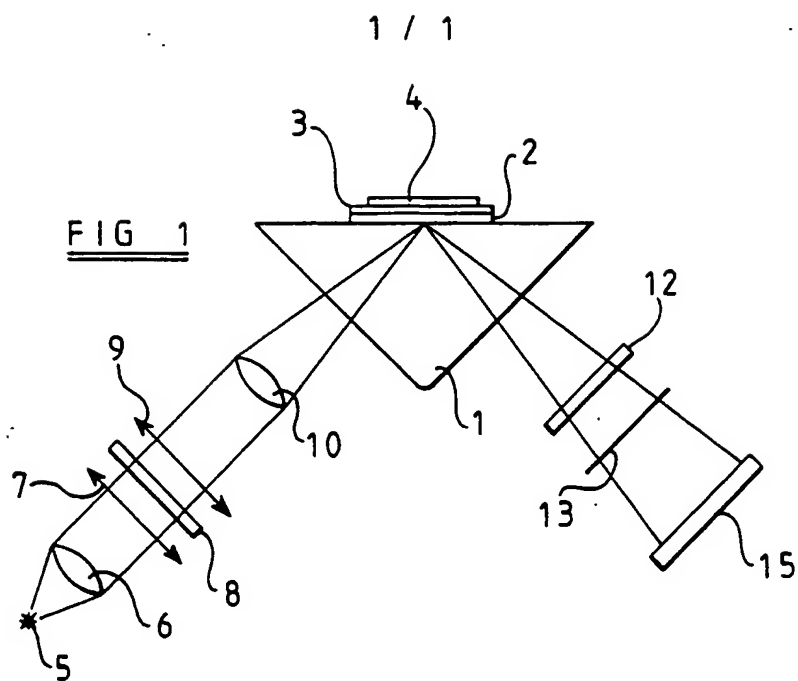
- a) irradiating a resonant optical sensor with polychromatic light from a light source at a range of angles simultaneously,
- b) applying to the incident light a modulation as a function of frequency,
- c) permitting light reflected from the sensor to reach a detector only when resonance occurs,
- d) contacting the sample with the sensor, and
- e) monitoring the intensity of the reflected light reaching the detector as a function of the angle of incidence.

14. A method as claimed in Claim 13, comprising the further

step of fitting the measured intensities to a known function.

15. A method as claimed in Claim 13, comprising the further step of subjecting the measured intensities to a Fourier Transformation.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N21/55

According to International Patent Classification (IPC) or to both national classification and IPC:

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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 04617 (FISONS) 19 March 1992 cited in the application see page 2, paragraph 3 see page 5, paragraph 3 see page 7, paragraph 5 - page 8, paragraph 2 see claims 1,2,7,8,11; figures 1,2	1,9-13
A	WO,A,92 03720 (FISONS) 5 March 1992 see page 5, paragraph 3 - page 7, paragraph 2 see page 8, paragraph 2 see page 10, paragraph 2	1,6-8, 12,13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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European Patent Office, P.O. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Krametz, E

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information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9204617	19-03-92	EP-A- 0546061	16-06-93
WO-A-9203720	05-03-92	EP-A- 0543831	02-06-93
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